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Toxic and Carcinogenic Effects of Solid Particles in the Respiratory Tract

D.L. Dungworth J.L. Mauderly G. Oberdörster
Editors

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Foreword

The series of International Inhalation Symposia, started in 1987, is held biennially in Hannover. Sponsored by the International Life Sciences Institute (ILSI), Hannover Medical School, and the Fraunhofer Institute of Toxicology and Aerosol Research, the symposia present and discuss subjects of current importance in the field of inhalation toxicology. They bring together scientists from government, academia, and industry, offering an opportunity for opinions to be heard, questions raised, and solutions sought.

THE EDITORS
U. Mohr
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Series Foreword

The International Life Sciences Institute is a nonprofit, worldwide foundation established in 1978 to advance the understanding of scientific issues relating to toxicology, nutrition, food safety, and the environment and to promote agreement and regulatory harmonization in these areas. By bringing together scientists from academia, government, and industry, ILSI seeks a balanced approach to solving problems with broad implications for the well-being of the general public.

ILSI is affiliated with the World Health Organization as a nongovernmental organization and has specialized consultative status with the Food and Agriculture Organization of the United Nations. Headquartered in Washington, D.C., ILSI has branches in Argentina, Australasia, Brazil, Europe, Japan, Mexico, North America, and Southeast Asia, with branches under consideration elsewhere. In addition, ILSI recently established a focal point in Beijing, China.

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Histopathological Findings in the Rat and Hamster Respiratory Tract in a 90-Day Inhalation Study Using Fresh Sidestream Smoke of the Standard Reference Cigarette 2R1

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Introduction

While histopathological changes in the respiratory tract of rats and hamsters in short-term and long-term inhalation studies with mainstream smoke (MS) have been reported extensively in literature, there are only three published inhalation studies on rats and on rats and hamsters with sidestream smoke in which histopathology is the main end point. In these studies, the findings observed were epithelial hyperplasia and squamous metaplasia in the rostral nose of the rat at a concentration of 4 µg/L (von Meyerinck et al. 1989) and epithelial hyperplasia in the rostral nose of the rat at a concentration of 10 µg/L (Coggins et al. 1992, 1993). No findings were seen in the rat larynx. In the hamster, no histopathological changes were observed in the respiratory tract (von Meyerinck et al. 1989).

Methods and Evaluations

Animals and Housing

Male Sprague Dawley rats, Crl:CDBR (Charles River, Germany), and male Syrian golden hamsters, Lak:LVG(SYR) (Charles River, U.S.A.), were used, the body weight at the start of the inhalation period being approximately 200 and 80 g, respectively. The animals were housed under standardized conditions (room temperature 22 ± 1 °C, relative humidity 55 ± 10%, and light–dark cycle 14.5 hours:9.5 hours) in polycarbonate cages, type 3, with granulated dust-free wood as bedding material. Diet and drinking water were supplied ad libitum except during exposure.

Animal Exposure

The animals were nose-only-exposed to fresh sidestream smoke (FSS) for 7 hours/day, 7 days/week for 90 days in glass tubes adapted to the shape of the skull at the front end and sealed with rubber stoppers at the rear end. The TPM concentrations in the FSS of 2 and 6 μg/L are significantly above the levels reached in occupied spaces with smoking (U.S. EPA 1992). The air flow rate in the exposure chamber, cross section 0.1 m × 0.1 m, was 50 L/minute. Sham-exposed animals served as controls.

Group Size

Twenty male rats and 20 male hamsters per group were allocated to one sham exposure group and two FSS groups. Of these, 10 rats and 10 hamsters per group were kept for a 21-day postinhalation period.

Generation of Sidestream Smoke

University of Kentucky standard reference cigarettes 2R1 were smoked on automatic 30-port positive pressure smoking machines (mean puff volume, 35 mL; puffs/cigarette, 9.8; puff frequency/cigarette, 1/minute; puff duration, 2 seconds). The resulting sidestream was collected using a circular hood inside the smoking machine. The maximum age of the smoke was approximately 7 seconds. The two FSS concentrations were obtained by dilution with particle-filtered air.

Characterization of Test Atmosphere

Relevant analytical parameters were determined at appropriate intervals to characterize the FSS and the air used for sham exposure as well as to check the reproducibility of the FSS generation (Table 1).

Carboxyhemoglobin

The steady-state proportion of carboxyhemoglobin was determined at the end of daily exposure three times during the inhalation period to confirm smoke exposure. In the low- and high-FSS groups it was 1.6 and 3.7% for the rats and 2.0 and 4.5% for the hamsters, respectively.

Biological Parameters

The primary parameters were gross pathology and histopathology of the respiratory tract as well as morphometrical determination of the laryngeal epithelial thickness. HE-stained paraffin sections cut at defined levels (Young

Table 1. Concentrations of relevant analytical parameters.

	FSS group	
Analytical parameter	Low	High
TPM (μg/L)	2.1	6.0
CO (ppm)	9	22
Nicotine (μg/L)	0.6	1.3
Ammonia (μg/L)	1.1	2.6
Formaldehyde (ppm)	0.19	0.38
Acetaldehyde (ppm)	0.24	0.55
Acrolein (ppm)	0.03	0.07

1981, Lewis 1980, Lamb and Reid 1969) were evaluated semiquantitatively and morphometrically. Secondary parameters were in-life observations, mortality, body weight, and organ weights.

Results

In both species, no smoke-exposure-related effects were seen for in-life observations, mortality, body weight, organ weights, and gross pathology. The histopathological findings observed in rats were as follows:

nose (rostral): reserve cell hyperplasia of the respiratory epithelium
larynx
  base of epiglottis: hyperplasia of squamous epithelium
  arytenoid projections
    ventral depression: hyperplasia of cuboidal epithelium
    floor of the larynx: squamous metaplasia of the pseudostratified epithelium
    vocal cords:
      lower medial surface: hyperplasia of the squamous epithelium (Figures 1–3)
      upper medial surface: squamous metaplasia of the pseudostratified epithelium (Figures 4–6)
  vocal folds: hyperplasia of the squamous epithelium

The severity of these findings in rats was slight, and they were observed mainly in the high FSS concentration group. No smoke-exposure-related histopathological changes were observed in trachea and lungs. A dose-dependent increase in epithelial thickness in the larynx compared to sham was observed at the floor of the larynx and at the lower medial surface of the vocal

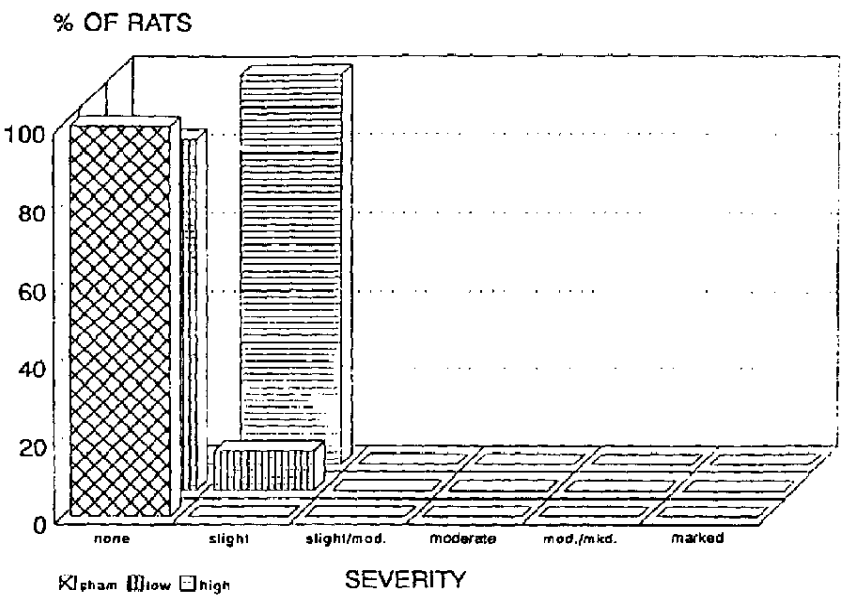

Figure 1. Distribution of hyperplasia of squamous epithelium at the arytenoid projections, vocal cords, lower medial surface, at the end of a 90-day inhalation period.

cords; the increase at the respective sites was 19 and 32% in the 6 µg/L TPM concentration group and was statistically significant. All the aforementioned findings reversed completely during the 21-day postinhalation period, the exception being hyperplasia at the vocal cords, which was still present in three rats of the high FSS concentration group. All findings are considered to be an adaptive response to repeated irritation.

In the hamster respiratory tract, no histopathological changes were seen.

Conclusion

The reserve cell hyperplasia of the rat nasal respiratory epithelium and the lack of findings for the hamster are in accordance with published literature (von Meyerinck et al. 1989, Coggins et al. 1992, 1993). The slight hyperplasia and the slight squamous metaplasia found in the rat laryngeal epithelium have not been reported to date in the literature. The changes were reversible and are considered to be an adaptive response to repeated irritation.

The No Observed Effect Level (NOEL) for all FSS-related findings for this study is between 2 and 6 µg TPM/L for rats. This concentration range is between 1 and 2 orders of magnitude above the average environmental concentration.

Figure 2. Transverse section at the arytenoid projections, vocal cords, lower medial surface: sham-exposed rat showing normal epithelium; H & E × 135.

Figure 3. Transverse section at the arytenoid projections, vocal cords, lower medial surface: high-dose SS-exposed rat showing hyperplasia of squamous epithelium; H & E × 132.

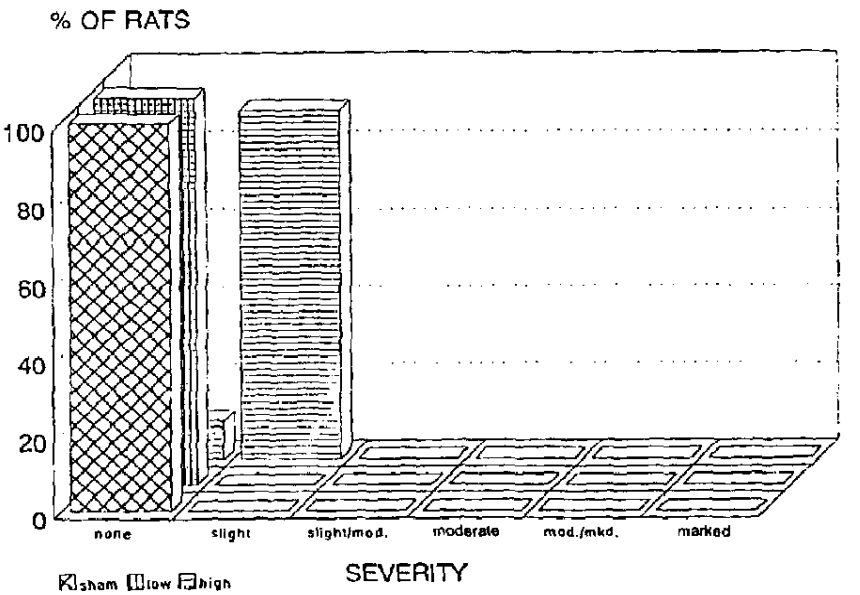

Figure 4. Distribution of squamous metaplasia at the arytenoid projections, vocal cords, upper medial surface, at the end of a 90-day inhalation period.

Figure 5. Transverse section at the arytenoid projections, vocal cords, upper medial surface: sham-exposed rat showing normal epithelium; H & E × 132.

Figure 6. Transverse section at the arytenoid projections, vocal cords, upper medial surface: high-dose SS-exposed rat showing squamous metaplasia; H & E × 135.

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